Bulletin of the Japanese Society of Scientific Fisheries 48(12), 1775–1782 (1982)

Improvement of Dietary Value of Brine Shrimp *Artemia salina* for Fish Larvae by Feeding Them on ω3 Highly Unsaturated Fatty Acids*1

Takeshi WATANABE,*2 Masahiro OHTA,*2 Chikara KITAJIMA,*3 and Shrio FUJITA*3

(Received May 13, 1982)

Experiments were conducted in order to improve the dietary value for marine fish juveniles of *Artemia* nauplii of the freshwater type, high in 18: 3ω3, by allowing them to feed directly on ω3 HUFA. In this method lipids containing ω3 HUFA were given directly to *Artemia* nauplii by homogenizing lipid with a small amount of raw egg yolk and water, together with baker's yeast; an emulsion resulted. The dietary value of the nauplii for juveniles of flounder, rock sea bream and red sea bream was compared for fish fed on various kinds of emulsified lipids or on baker's yeast.

The newly-hatched nauplii were found to take up lipids very easily by this method. Feeding on the newly-hatched nauplii of the freshwater type or on those fed respectively baker's yeast and corn oil resulted in low growth and survival in all fish species used and in addition the rate of normal fish in the activity test was especially low. The dietary value of the nauplii was found to be effectively improved by feeding them lipids containing high amounts of ω3 HUFA such as cuttlefish liver oil, demonstrating that the class of EFA contained in *Artemia* is the principal factor in the food value of Artemia nauplii to fish.

Artemia eggs and nauplii from different locations (California, Brazil, Australia and China), were also analyzed for fatty acids in order to compare their food values to fish.

The nauplii of *Artemia salina* have been widely used as a food in rearing marine fish larvae and crustaceans. However, it has been observed that a single dosage of *Artemia* nauplii frequently resulted in a severe lethargy of the larvae, and a high mortality was observed in various kinds of marine fish after they were fed on nauplii for a period of 1 or 2 weeks,[1-8] although this depended upon the fish species as well as the place of production of *Atremia*. Some species of flounder, one species of mullet *Liza haematocheila,* one salmonoid *Plecogrossus altivelis,* and some gobiid fish were not easily affected, but in contrast the larvae of the yellowtail *Seliola quinqueradiata* were very susceptible to this phenomenon.[3,4] In addition, many investigators have reported a heavy loss of larvae of prawns, crabs, and marine fish fed on *Artemia* nauplii from Utah.[5-9]

BOOKHOUT and COSTLOW[8] reported that the difference in mortality and survival rate of the developmental stages of four crab species may be attributed to the difference of the DDT content in the *Artemia* nauplii from California and Utah. DDT was reported to be approximately three times higher in the latter. WICKENS[9] mentioned that *Artemia* eggs and nauplii from San Francisco and Utah were analyzed for the presence of pesticides, heavy metals, carotenoids, sterols, and fatty acids. Although some difference were found, none of them could be confidently labeled as the cause of the poor nutritional value of the Utah *Artemia* nauplii.

Recently WATANABE *et al.*[10] have demonstrated that *Artemia* obtaihed from different locations could be classified into two types by the fatty acid composition; one (the freshwater type) contained a high proportion of 18: 3ω3, which is the essential fatty acid (EFA) for freshwater fish; the other (the marine type) had a high content of 20: 5ω3, which is one of the EFA for marine fish. Furthermore, *Artemia* of the marine type were found to be satisfactory as a food for juvenile red sea bream, *Pagrus major* (TEMMINCK and SCHLEGEL), and the dietary value of the nauplii was improved by feed-

*1 Nutritional Studies in the Seed Production of Fish—XII.

*2 Laboratory of Fish Nutrition, Tokyo University of Fisheries, Konan 4, Minato, Tokyo 108, Japan (渡辺武・太田雅大: 東京水産大学水族栄養学講座).

*3 Aqualture Research Laboratory, Nagasaki Prefectural Institute of Fisheries, Nomozaki, Nagasaki 851-05, Japan (北島 力・藤田矢郎: 長崎県水産試験場).

ing them marine *Chlorella* and baker's yeast supplemented with cuttlefish liver oil (ω-Yeast).[11] Both contain substantial amounts of the EFA required by marine fish.[12] These results suggested that the class of EFA contained in *Artemia* is the principal factor in the food value of *Artemia* to fish.

Later experiments by WATANABE *et al.*[13] have clarified the relationship between the dietary value of *Artemia* and their content of ω3 highly unsaturated fatty acids (ω3 HUFA), particularly 20: 5ω3 and 22: 6ω3.

This study was conducted in order to improve the dietary value for marine larval fish of *Artemia* nauplii of the freshwater type by allowing the nauplii to feed on ω3 HUFA by the direct method. In this method, in contrast with the indirect method using ω-Yeast, lipids containing ω3 HUFA are given directly to *Artemia* nauplii by homogenizing lipid with samll amount of raw egg yolk and water, resulting in a stable emulsion, which is fed together with bakers' yeast. *Artemia* eggs and nauplii from different locations were also analyzed for fatty acids in order to compare their food value to fish.

Materials and Methods

Artemia salina

The eggs and nauplii of *Artemia salina* obtained from California (San Francisco), Brazil, Australia, and China (Tien-tsin) during 1979–1981 were analyzed for fatty acids by the methods described previously.[14–16] *Artemia* cysts were well ground in a glass mortar and pestle and extracted twice with a 20-fold volume of chloroform: methanol (2: 1).[17]

As a preliminary step, the fatty acid compositions of three lots of eggs from San Francisco were checked by GLC, and those *Artemia* eggs containing a high proportion of 18: 3ω3 were hatched in vigorously aerated sea water in a 100 *l* tank held at 24–26°C in all the experiments. After 2 days the nauplii which had hatched were separated and were then washed in clean sea water.

Feeding of Artemia with Lipids by the Direct Method

The newly-hatched nauplii were cultured in 30 *l* tanks containing filtered sea water at 24–26°C, aerated vigorously, and fed respectively with various kinds of emulsified lipids once a day. In this method 1.5 g of lipid was emulsified with 0.3 g of raw egg yolk and 20 m*l* of sea water by homogenizing with a mixer for 3 min, and was given directly to *Artemia* nauplii in a 30 *l* tank, together with the same weight of baker's yeast as the nauplii in the tank. After a 15–19 h feeding they were washed with clean water before feeding to fish.*

Feeding of Fish Larvae with Artemia

Feeding experiments were conducted four times (Experiments I, II, III and IV) to compare the dietary value of *Artemia* nauplii fed with or without emulsified lipids to juveniles of flounder *Paralichthys olivaceus*, rock sea bream *Oplegnathus fasciatus* and red sea bream, at the Aquaculture Reasearch Laboratory of the Nagasaki Prefectural Institute of Fisheries.

1. Experiment I (Flounder) The juveniles of 7.06 mm in body length, which had been fed on rotifers *Brachionus plicatilis* cultured with both marine *Chlorella* and ω-Yeast, were randomly divided into 3 lots of 500 fish each in a 100 *l* tank. Each experimental group was fed with one variety of the nauplii fed for 18–19 h on the emulsified cuttlefish liver oil, or corn oil, or those without treatment (Control), respectively for 19 days at water temperature of 12.6–21.4°C (average 16.9°C) under conditions of aeration at 300 m*l*/min. The amount of water supplied to each tank was 400–500 m*l*/min and half of the water in each tank was changed daily for removal of residual nauplii.

2. Experiment II (Rock sea bream) The juvenile rock sea bream, 9.7 mm in body length and 9.0 mg in body weight, which had been fed on rotifers, *Tigriopus* sp. and minced fish meat, were divided into 5 lots of 100 fish each in 30 *l* tanks. Each lot was then fed respectively on one variety of the nauplii cultured separately for 15–19 h with ω-Yeast, with baker's yeast, or with an emulsion of cuttlefish liver oil plus baker's yeast, or the nauplii without feeding (Control), or *Tigriopus*, for 10 days at water temperature of 23.1–24.9°C under conditions of aeration at 200 m*l*/min. The amount of water supplied to each tank was 160 m*l*/min. The amount of the nauplii and *Tigriopus* given to the fish was 7.2 g and 3.2 g per day, respectively.

3. Experiment III and IV (red sea bream) Feeding experiments were conducted twice (Expts. III and IV) by using red sea bream juveniles, which had been fed on rotifers cultured with both marine *Chlorella* and ω-Yeast, and *Tigriopus*. The

* T. WATANABE, T. TAMIYA, A. OKA, C. KITAJIMA, and S. FUJITA: Oral presentation at the annual meeting of Japan. Soc. Sci. Fish., in Mie, October 1981.

juveniles were divided into 6 lots of 350 fish each in Exp. III and 7 lots of 350 fish each in Expt. IV. In Expt. III fish were fed as shown in Table 6, on the nauplii cultured with emulsified lipids, respectively corn oil, pollock liver oil, cuttlefish liver oil, or a mixture of ω3 HUFA containing 53.0% of 20: 5ω3 and 20.7% of 22: 6ω3, all including baker's yeast, or those fed on baker's yeast alone, or on *Tigriopus*.

In Expt. IV the feeding experiment with red sea bream was conducted by feeding them one variety of the nauplii shown in Table 7. In both experiments the amount of *Artemia, Tigriopus* and rotifers given to fish was 20 g per day.

Results and Discussion

Fatty Acid Composition of Artemia from Different Locations

Artemia eggs and nauplii from different locations (California, Brazil, Australia and China) were analyzed for fatty acids in order to clarify the food value of particular *Artemia* nauplii to fish. As summarized in Tables 1, 2 and 3, the results were found to be very similar to those of the previous study[10,13] and confirmed that *Artemia* can be roughly classified into two types, the freshwater type and the marine type.

1. San Francisco As already pointed out in the previous papers, *Artemia* eggs from San Francisco differed quite markedly from year to year or lot to lot (Table 1), although the origin of strain and the place of production were unknown. They were found to consist mainly of the types, high 18: 3ω3 together with 18: 4ω3 (eggs D and E in 1980, eggs B and C in 1981) or high in 20: 5ω3 (egg A in 1979), but some lots were low in both 18: 3ω3 and 20: 5ω3 (eggs B and C in 1980). In both the freshwater and marine types the proportion of 18: 2ω6 was high and in addition 20: 4ω6 was important in the latter. *Artemia* which do not belong to these two types were low in the percentage of 18: 2ω6 and high in the content of 16: 0.

2. Brazil and Australia The results of analyses are shown in Table 2. The eggs and nauplii from Brazil in 1980 were found to be characteristically low in the content of 18: 3ω3, together with 18: 4ω3, and high in the proportion of ω6 fatty acids such as 18: 2ω6 and 20: 4ω6. Eggs A and B were of a middle type, and eggs C-F belonged to the marine type, being high in both 20: 5ω3 and 18: 2ω6. Eggs D were found to be the highest in the percentage of 22: 6ω3 among *Artemia* hiterto examined.

Artemia from Australia in 1980 were of the typical marine type.

3. China As reported previously,[13] *Artemia*

Table 1. Certain fatty acids of total lipids in *Artemia* eggs and nauplii from San Francisco

Fatty acid	1979	1980								1981		
	A	A	B	C	D	E	F*1	G*1	H*1	A	B	C
14: 0	2.3	3.5	2.9	3.6	1.3	2.1	2.2	0.6	0.7	1.6	1.1	0.7
16: 0	13.3	26.6	25.3	25.9	14.9	23.7	9.2	11.0	12.2	15.2	13.6	10.6
16: 1ω7*2	16.4	16.3	15.7	12.9	5.5	7.4	14.8	3.8	10.4	10.5	4.3	5.4
18: 0	2.4	5.1	5.1	3.7	3.5	4.1	2.0	3.3	3.2	2.9	3.2	3.0
18: 1ω9*2	28.2	25.8	27.6	19.8	28.0	23.7	19.1	26.7	34.9	28.4	27.1	26.3
18: 2ω6	8.3	2.6	2.9	2.5	6.3	5.4	8.3	8.9	6.6	7.1	6.1	7.6
18: 3ω6	0.9	0.5	0.4	0.6	—	—	1.2	—	0.4	0.6	—	0.8
18: 3ω3	2.3	3.3	4.2	4.8	22.4	14.7	5.4	27.6	17.2	17.2	28.1	27.0
18: 4ω3	0.2	0.3	0.9	1.1	3.2	3.4	0.6	6.0	2.5	2.7	3.6	5.2
20: 1	0.1	0.9	1.2	1.1	0.3	1.0	tr	0.5	0.5	0.4	0.4	0.5
20: 2ω6	0.1	1.2	1.2	1.0	0.3	0.6	tr	0.1	0.1	—	—	0.1
20: 4ω6	7.7	2.7	0.7	0.6	0.8	0.8	3.5	1.1	1.4	1.4	0.7	1.3
20: 4ω3	—	0.1	tr	tr	0.2	0.5	0.3	0.9	0.3	0.4	—	0.9
20: 5ω3	7.5	3.9	1.7	0.9	2.7	0.6	6.8	0.3	3.5	3.6	2.4	2.1
22: 1	—	0.7	0.5	0.3	0.6	0.3	tr	—	0.3	—	0.2	tr
22: 4ω6	—	0.3	0.2	0.4	—	—	0.2	0.1	—	—	—	0.2
22: 6ω3	0.1	0.4	0.1	0.2	0.1	0.1	0.2	—	tr	—	—	—
Σω3 HUFA*3	7.6	4.4	1.8	1.1	3.0	1.2	7.3	1.2	3.8	4.0	2.4	3.0
Lipid %	2.9	—	—	—	—	—	1.9	3.9	2.3	—	—	3.5

*1 Nauplii just after hatching. *2 Small amounts of the other monenes were included. *3 C_{20}: $_3$<ω3 fatty acids.

Table 2. Certain fatty acids of total lipids in *Artemia* eggs from Brazil and Australia in 1980

Fatty acid	Brazil 1980							Australia 1980
	A	B	C	D	E	E*1	F	
14:0	3.3	3.4	2.1	3.6	2.4	1.7	2.0	1.6
16:0	16.0	18.2	13.7	18.0	14.7	12.2	13.7	13.9
16:1ω7*2	18.6	14.4	13.8	14.6	14.7	12.8	14.1	9.9
18:0	1.9	2.9	3.2	2.8	2.7	4.1	2.6	2.8
18:1ω9*2	21.8	23.7	28.9	16.2	26.6	30.7	28.3	33.3
18:2ω6	7.2	6.4	8.5	3.1	7.7	9.3	11.8	5.2
18:3ω6	1.9	3.2	0.8	5.1	—	—	—	0.1
18:3ω3	3.3	1.1	3.2	0.9	3.6	3.3	2.7	10.1
18:4ω3	0.3	tr	0.6	tr	0.7	0.4	0.9	3.8
20:1	0.9	1.2	0.4	1.9	0.5	0.5	0.3	0.4
20:2ω6	0.5	0.3	0.1	0.2	0.1	—	—	0.2
20:4ω6	2.7	3.2	4.5	3.2	4.0	4.6	3.6	1.1
20:4ω3	0.1	tr	0.4	tr	0.3	0.4	0.2	1.0
20:5ω3	3.9	3.5	5.9	4.6	5.8	6.5	5.8	8.6
22:1	0.7	1.0	0.4	1.8	0.5	—	—	tr
22:4ω6	0.4	0.5	0.1	0.9	—	—	—	tr
22:6ω3	0.4	0.6	tr	1.6	0.1	—	0.2	0.2
Σω3 HUFA*3	4.4	4.1	6.3	6.2	7.2	6.9	6.2	9.8
Lipid %	—	—	5.4	—	—	—	—	7.9

*1 Nauplii from egg E. *2 Small amounts of the other monoenes were included. *3 $C_{20:3}<\omega3$ fatty acids.

Table 3. Certain fatty acids of total lipids in *Artemia* eggs from Tien-tsin during 1979 to 1981

Fatty acid	1979					1980			1981
	A*1	B*1	A	B	C	A	B	C	A
14:0	0.9	0.8	3.0	2.8	2.0	5.0	5.5	2.1	2.0
16:0	9.7	9.3	12.1	12.7	12.7	23.0	21.2	12.5	13.1
16:1ω7*2	13.6	13.4	22.6	24.0	22.4	24.7	22.8	20.1	19.1
18:0	6.0	6.0	3.5	2.9	3.3	4.4	3.8	3.2	3.3
18:1ω9*2	33.5	33.8	26.1	20.2	28.3	22.1	17.4	24.9	25.3
18:2ω6	4.4	4.4	4.1	3.8	4.3	1.6	2.2	4.2	5.0
18:3ω3	5.3	5.1	5.5	6.0	5.1	0.4	0.6	6.4	6.6
18:4ω3	0.6	0.6	0.9	1.0	0.7	0.4	0.9	1.0	1.3
20:1	0.7	0.7	0.2	0.4	—	1.7	1.9	0.4	0.4
20:4ω6	2.8	3.0	1.2	1.1	1.5	0.8	0.7	1.8	1.4
20:4ω3	0.7	0.7	—	—	—	—	—	0.2	0.1
20:5ω3	13.0	13.2	9.2	10.2	11.3	1.9	1.3	10.9	9.3
22:1	—	—	—	—	—	0.6	0.6	tr	0.7
22:6ω3	—	—	—	—	—	0.2	0.3	tr	—
Σω3 HUFA	13.7	13.9	9.2	10.2	11.3	2.1	2.2	11.1	9.4
Lipid %	4.2	4.4	3.7	3.9	5.2	—	—	9.3	2.5

*1 Nauplii from egg A and B. *2 Small amounts of the other monenes were included.

Table 4. Improvement of dietary value of *Artemia* for flatfish by the direct method (Exp. I)*1

Lipid given to *Artemia*	ω3 HUFA in *Artemia* (%)	Total body length (mm)		Average body wt (mg)	Survival rate (%)	Normal fish in activity test (%)
		Initial	Final			
Cuttlefish liver oil	0.40	7.36	12.36	13.7	67.6	80.0
Control*2	0.05	7.30	11.15	9.8	35.6	13.3
Corn oil	0.05	7.16	9.86	5.8	27.1	0

*1 Feeding period was 19 days.
*2 Nauplii just after hatching (48 h).

eggs from Tien-tsin belong to the marine type, but some of those in 1980 (eggs A and B) did not belong to any type, being low in both ω3 and ω6 fatty acids and high in 16: 0 (Table 3). The other eggs in 1979, 1980 and 1981 were all of the typical marine type. After hatching out the percentage of 20: 5ω3 and 18: 1ω9 increased and that of 16: 0 and 16: 1ω7 decreased.

Feeding of Flounder with Artemia in Expt. I

The results of the comparison of dietary value of the newly-hatched nauplii with those fed on the emulsified cuttlefish liver oil or corn oil are shown in Table 4 and Fig. 1. During the experimental period the nauplii fed on various lipids were analyzed for fatty acids three times and were found to incorporate lipids effectively by the direct method. The ω3 HUFA content increased to an average of 0.40% from the initial value of 0.05% in the nauplii fed on cuttlefish liver oil and was as low as 0.05% in those fed on corn oil or in the intact nauplii. In Expt. I, feeding with newly-hatched nauplii, and those fed on corn oil, both resulted in heavy losses of the fish at around 13th day of feeding and the cumulative mortality of the groups increased in proportion to the feeding period, reaching 64.4 and 72.9% respectively at the end of the experiment. Furthermore, the growth and activity of these two lots of fish were also low (Fig. 2). The percentage of fish showing abnormal behavior in the activity test at the termination of the feeding experiment was significantly higher. For this test 30–50 fish were dipped out of water with a scoop net, held for 5 s, and moved to a 30 *l* tank for a check of fish activity.

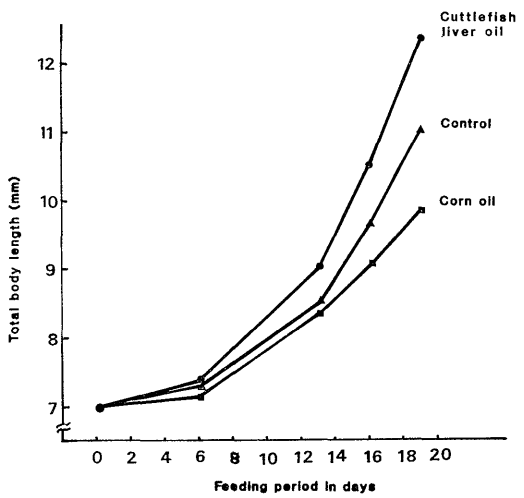

Fig. 1. Growth-response curves of juvenile flounder fed on the intact nauplii (Control) and those cultured with cuttlefish liver oil and corn oil in Experiment I.

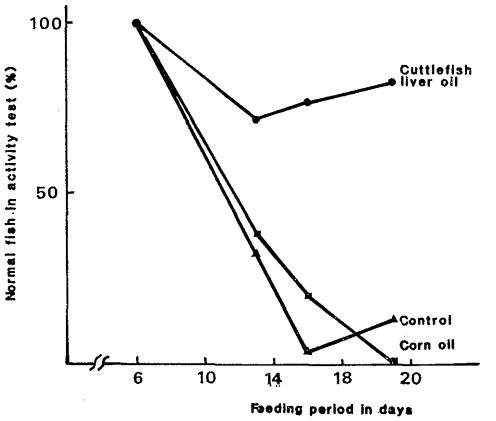

Fig. 2. Changes in the proportion of normal fish in the activity test during the feeding period in Experiment I.

The dietary value of the nauplii was effectively improved by feeding them cuttlefish liver oil containing a high amount of ω3 HUFA and the high mortality was markedly reduced in the fish fed these nauplii. In red sea bream[18] the most marked difference between the fish fed respectively the newly-hatched nauplii from San Francisco, and the nauplii containing ω3 HUFA, was the shock syndrome observed in the fish fed on the former nauplii during the activity test. This was not observed in flounder in this experiment. This may indicate that flounder posses slightly stronger resistance against EFA-deficiency than red sea bream.

Feeding of Rock Sea Bream with Artemia in Expt. II

The results of 10 days of feeding in rock sea bream are shown in Table 5 and Fig. 3. The ω3 HUFA content in the nauplii fed on ω-Yeast and cuttlefish liver oil was about 0.3%, slightly lower than that determined in Expt. I, but that in those fed on baker's yeast or without feeding was less than 0.1%. *Tigriopus* was found to contain 0.4% ω3 HUFA, relatively lower than that used in the later experiment.

The fish fed on the nauplii cultured with ω-Yeast or emulsified cuttlefish liver oil, which containing a relatively high amount of ω3 HUFA, showed good results in growth, survival and activity test, as observed in flounder. On the other hand, in the fish fed on the intact nauplii or those fed on baker's yeast, both low in the ω3 HUFA

Table 5. Improvement of dietary value of *Artemia* for rock sea bream by the direct method (Expt. II)*3

Feed given to *Artemia*	ω3 HUFA in *Artemia* (%)	Total body*1 length (mm)		Average body*1 weight (mg)		Survival rate (%)	Normal fish in activity test (%)
		Initial	Final	Initial	Final		
ω-Yeast	0.30	9.7	20.4	9.0	145.1	78.3	86.7
Cuttlefish liver oil	0.31	9.7	20.3	9.0	142.9	81.4	100
Baker's yeast	0.08	9.7	19.3	9.0	117.2	41.4	3.4
Control*2	0.10	9.7	19.5	9.0	124.8	59.2	10.0
Tigriopus	0.40	9.7	19.4	9.0	123.0	77.1	100

*1 Average values of 30 fish. *2 Nauplii just after hatching. *3 Feeding period was 10 days.

Table 6. Improvement of dietary value of *Artemia* for red sea bream by the direct method (Expt. III)*1

Feed given to *Artemia*	ω3 HUFA in *Artemia* (%)	Total body*2 length (mm)		Average body*2 weight (mg)		Survival rate (%)	Normal fish in activity test (%)
		Initial	Final	Initial	Final		
Baker's yeast	0.12	14.7	22.0	35.2	151.9	58.9	23.0
Corn oil	0.03	〃	22.6	〃	158.0	52.3	31.5
Pollock liver oil	0.21	〃	23.7	〃	188.9	76.3	86.5
Cuttlefish liver oil	0.77	〃	23.6	〃	182.5	83.1	99.6
Methyl ω3 HUFA	0.71	〃	23.4	〃	178.7	72.0	99.3
Tigriopus	0.50	〃	22.6	〃	181.0	89.1	100

*1 Feeding period was 9 days. *2 Average values of 50 fish.

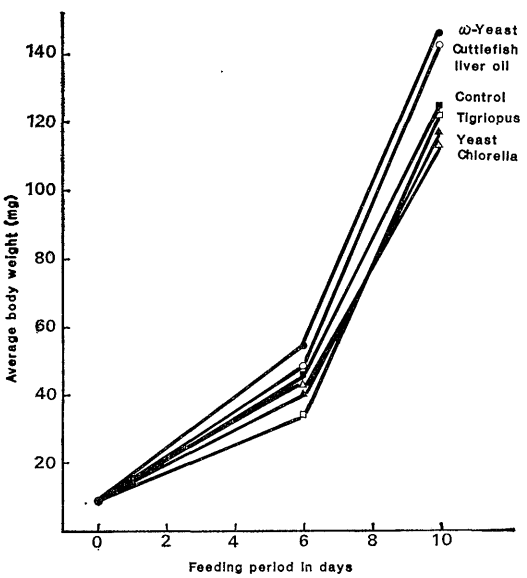

Fig. 3. Growth-response curves of rock sea bream juveniles fed on *Tigriopus* or the newly-hatched nauplii and those receiving ω-Yeast and cuttlefish liver oil, respectively in Experiment II

content, the rate of growth and survival was low and the percentage of normal fish in the activity test was as low as 10.0 and 3.4%, respectively. The fish fed on *Tigriopus* in place of *Artemia* also showed good results, although the growth rate was relatively lower because a lower amount of *Tigriopus* was given to the fish. The survival rate of *Artemia* nauplii during the cultivation with ω-Yeast, cuttlefish liver oil and baker's yeast was 99.7, 73.9 and 94.5%, respectively.

Feeding of Red Sea Bream with Artemia in Expts. III and IV

The results of 9 days of feeding red sea bream juveniles are shown in Table 6. The ω3 HUFA content of the nauplii fed on baker's yeast or corn oil was low, as was also observed in Expts. I and II, and was increased to 0.21% when they were fed emulsified pollock liver oil. Fatty acids of the nauplii fed on cuttlefish liver oil or methyl esters of a ω3 HUFA mixture were found to contain a high amount of ω3 HUFA, resulting in 0.77 and 0.71% ω3 HUFA respectively in the

Table 7. Improvement of dietary value of *Artemia* for red sea bream juveniles by the direct method (Expt. IV)*1

Feed given to *Artemia*	ω3 HUFA in *Artemia* (%)	Total body*2 length (mm)		Survival rate (%)	Normal fish in activity test (%)
		Initial	Final		
Baker's yeast	0.09	8.9±0.7	12.5±2.6	32.0	21.4
Corn oil	0.07	〃	12.2±2.2	35.7	15.2
Pollock liver oil	0.15	〃	13.4±2.2	39.7	56.7
Polloek & Cuttlefish liver oil (1:1)	0.30	〃	13.1±1.3	53.4	67.9
Cuttlefish liver oil	0.33	〃	13.3±2.3	63.1	98.2
Methyl ω3 HUFA	1.01	〃	14.1±2.1	52.0	96.7
Rotifer	—	〃	11.0±0.8	81.7	98.3

*1 Average values of 30 fish. *2 Feeding period was 7 days.

Table 8. Certain fatty acids of total lipids from red sea bream juveniles fed respectively rotifers and the nauplii cultured with baker's yeast or fed on various kinds of lipids in Experiment IV

Fatty acid	Red sea bream juveniles fed						
	Artemia nauplii cultured with						Rotifer
	Baker's yeast	Corn oil	Pollock liver oil	Pollock & cuttlefish liver oil	Cuttle-fish liver oil	Methyl 3 HUFA	
16:0	19.3	21.0	20.1	21.0	24.9	24.7	32.8
16:1ω7*1	6.2	6.8	6.7	5.3	5.6	5.8	12.3
18:0	10.5	7.7	9.6	10.0	10.3	9.2	7.4
18:1ω9*1	27.5	23.4	26.8	22.6	22.1	19.8	12.4
18:2ω6	5.7	12.4	5.8	5.1	4.6	4.6	3.2
18:3ω3	7.3	7.0	6.3	5.2	4.7	5.7	0.3
18:4ω3	0.5	0.4	0.3	0.3	0.2	0.4	tr
20:1	1.2	0.8	3.1	1.8	1.7	0.6	1.5
20:3ω3 20:4ω6	4.2	4.2	2.9	4.5	3.8	3.8	4.3
20:4ω3	1.0	0.6	0.6	0.7	0.4	0.6	0.2
20:5ω3	3.6	3.3	3.2	6.6	6.1	8.2	5.0
22:1	0.8	0.4	1.8	0.6	0.8	0.9	0.5
22:5ω3	1.7	1.6	1.4	2.9	2.4	2.9	4.0
22:6ω3	2.1	2.1	1.6	6.0	5.8	5.3	5.9
Σω3 HUFA*2	8.4	7.6	6.8	16.2	14.7	17.0	15.1
Lipid %	2.9	3.1	2.6	2.8	2.6	2.6	2.6

*1 Small amounts of the other monoenes were included.
*2 $C_{20:3}$<ω3 fatty acids.

nauplii. These values were higher than those determined in Expts. I and II. This suggests that incorporation of lipids by nauplii depends upon the culture conditions such as water temperature and density, and activity of nauplii used. This has frequently been observed in the case of rotifers. The ω3 HUFA content in *Tigriopus* was 0.50%.

The juveniles receiving the nauplii fed on baker's yeast or corn oil showed low growth and survival rates and also low percentages of normal fish in the activity test. Elevation of the ω3 HUFA level in the nauplii by feeding them pollock liver oil, cuttlefish liver oil, or the mixture of ω3 HUFA, effectively improved these results, especially in the activity test, with results comparable to those obtained with *Tigriopus*. The survival rate of *Artemia* during cultivation with baker's yeast, corn oil, pollock liver oil, cuttlefish liver oil, or the ω3 HUFA mixture was respectively 93.9, 57.4, 69.3, 56.2 and 84.0%.

Results similar to those in Expt. III were also obtained in Expt. IV with red sea bream (Table 7). The growth and survival rates were low in the juveniles receiving the nauplii fed on baker's yeast or corn oil. Both rates were effectively improved by feeding the nauplii containing ω3

HUFA at more than 0.3%. Feeding rotifers cultured with both marine *Chlorella* and ω-Yeast also resulted in high rates of survival and normal fish in the activity test, although the growth rate was inferior to that in the other groups, since rotifers are slightly small for this size of fish.

As already demonstrated in the previous experiments,[10,18] the dietary value of *Artemia* is found to be effectively improved by elevating the ω3 HUFA content in *Artemia* by both the direct and indirect methods, indicating that the class of EFA contained in *Artemia* is the principal factor in the nutritional quality of *Artemia* to fish larvae. Thus the dietary value of *Artemia* of the freshwater type can be easily improved. In addition, it is also possible to further improve the dietary value of nauplii by allowing them to feed on fat-soluble vitamins together with other lipids, as demonstrated in rotifers by WATANABE *et al.**

Judging from the present results the nauplii containing ω3 HUFA at more than 0.3%, such as the nauplii of the marine type, may be satisfactory as a single feed for most fish, but it is thought that feeding them any type of lipid containing ω3 HUFA included by the direct or the indirect method may be effective. Since the lipid content of the nauplii gradually decreases after hatching.[10]

Fatty Acids of Red Sea Bream Fed Artemia in Expt. IV

The fatty acid compositions of total lipids in the red sea bream juveniles fed various nauplii or rotifers cultured with both marine *Chlorella* and ω-Yeast are shown in Table 8. As observed in the previous experiment,[18] the fatty acid compositions of the fish clearly reflect those of the nauplii and rotifers in a 7 day-feeding. The ω3 HUFA content was low in the fish fed on the nauplii cultured with baker's yeast or corn oil, and was about twice as high in the fish receiving the nauplii fed on cuttlefish liver oil, or the methyl esters of ω3 HUFA, or in those receiving rotifers. The fish receiving the nauplii fed on corn oil was high in the content of 18:2ω6 suggesting incorporation of this fatty acid by the nauplii from corn oil added to the culture medium.

Acknowledgement

We express here our sincere thanks to Prof. Dr. R. G. ACKMAN, Techincal University of Nova Scotia, Canada, who kindly read the manuscript and gave valuable suggestions. Thanks are also extended to Mr. A. ITO of Graduate Course, Tokyo University of Fiheries, for this technical assistance.

This study was supported in part by Grant-in Aid for Scientific Research of the Ministry of Education and in part by Research Fund of the Fisheries Agency.

References

1) T. FUSHIMI: *Bull. Hiroshima Pref. Fish. Exptr. St.*, **1**, 49–54 (1968).
2) R. HIRANO and Y. OSHIMA: *Bull. Japan. Soc. Sci. Fish.*, **29**, 282–297 (1963).
3) S. FUJITA: *Bull. Plankton Soc. Japan*, **20**, 49–53 (1973).
4) S. FUJITA: *Bull. Nagasaki Pref. Inst. Fish.*, **352**, 27–28 (1972).
5) L. B. SLOBODKIN: *Biol. Sci. Tokyo*, **18**, 16–23 (1968).
6) G. LITTLE: *Crustaceana*, **17**, 69–87 (1969).
7) M. R. REEVE: *Fishery Invest., Lond., Ser. II*, **26**, 38 (1969).
8) C. G. BOOKHOUT and J. D. COSTLOW: *Helgolander wiss. Meeresunters*, **20**, 435–442 (1970).
9) J. F. WICKENS: *J. exp. mar. Biol. Ecol.*, **10**, 151–170 (1972).
10) T. WATANABE, F. OOWA, C. KITAJIMA, and S. FUJITA: *Bull. Japan. Soc. Sci. Fish.*, **44**, 1115–1121 (1978).
11) O. IMADA, Y. KAGEYAMA, T. WATANABE, C. KITAJIMA, S. FUJITA, and Y. YONE: *Bull. Japan. Soc. Sci. Fish.*, **45**, 955–959 (1979).
12) Y. YONE: in "Yogyo to Shiryo Shishitsu" (ed. by Japan Sco. Sci. Fish.), Koseisha-Koseikaku, Tokyo, 1978, pp. 43–59.
13) T. WATANABE, F. OOWA, C. KITAJIMA, and S. FUJITA: *Bull. Japan. Soc. Sci. Fish.*, **46**, 35–41 (1980).
14) T. TAKEUCHI and T. WATANABE: *Bull Japan. Soc. Sci. Fish.*, **39**, 375–382 (1978).
15) M. MATSUI, T. WATANABE, and N. IKEKAWA: *Bull. Japan. Soc. Sci. Fish.*, **39**, 267–373 (1973).
16) T. TAKEUCHI and T. WATANABE: 投稿中
17) J. FOLCH, M. LEES, and G. H. S. STANLEY: *J. Biol. Chem.*, **226**, 497–507 (1959).

* See the footnote of page 1776.